

Measurement of heat production (thermogenesis) in cells using ERthermAC dye

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An abbreviated version of this protocol was published in Science Translational Medicine in Aug 2020

CRISPR-engineered human brown-like adipocytes prevent diet-induced obesity and ameliorate metabolic syndrome in mice

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Detailed protocol

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Materials

- BioTracker ERthermAC Temperature-Sensitive Live Cell Dye (SCT057, Merck)
(https://www.merckmillipore.com/TW/zh/product/BioTracker-ERthermAC-Temperature-Sensitive-Live-Cell-Dye_MM_NF-SCT057?ReferrerURL=https%3A%2F%2Ffbclid=IwAR1xNHOYdLL4iTg8IRPS0fHiwa88ZmXFFCu2vYnZTVg4ivkz3_YKd1AFb8)
- Prepare stock solution of ERthermAC dye (1 mM in DMSO)

Procedures

- Differentiate brown/white adipocytes in a black 96-well plate with clear bottom
- Prepare 250 nM ERthermAC dye in DMEM-H serum-free medium
- Add 100 μ L of staining solution per well
- Incubate 37°C for 30 min
- Wash with PBS twice
- Add 90 μ L of DMEM-H phenol red-free medium each well
- Equilibrate plate at 25 °C
- Measure 2-3 points of basal fluorescence (ex/em, 543/590) in a microplate reader
- Add PBS or 10 μ L of 10 μ M CL-316,243 (CL)/forskolin (Fsk) each well (final 1 μ M)
- Measure the fluorescence (ex/em, 543/590) every 5 min up to 1.5 ~ 2 hr.
- Calculate the decrease of fluorescence over time after drug treatment

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

- Wang, C. and Tseng, Y. (2020). Measurement of heat production (thermogenesis) in cells using ERthermAC dye. Bio-protocol Preprint. bio-protocol.org/prep525.
- Wang, C., Lundh, M., Fu, A., Kriszt, R., Huang, T. L., Lynes, M. D., Leiria, L. O., Shamsi, F., Darcy, J., Greenwood, B. P., Narain, N. R., Tolstikov, V., Smith, K. L., Emanuelli, B., Chang, Y., Hagen, S., Danial, N. N., Kiebish, M. A. and Tseng, Y. (2020). CRISPR-engineered human brown-like adipocytes prevent diet-induced obesity and ameliorate metabolic syndrome in mice . Science Translational Medicine 12(558). DOI: [10.1126/scitranslmed.aaz8664](https://doi.org/10.1126/scitranslmed.aaz8664)

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